Simultaneous Reverse Phase HPLC Estimation of Paracetamol and Rofecoxib in Tablets

G. SUBRAMANIAN, R. SHETTY, S. AGARWAL[1], S. PANDEY[1] AND N. UDUPA[1]*
Department of Quality Assurance, College of Pharmaceutical Sciences, MAHE, Manipal-576 104
[1]Dr. T. M. A. Pai Pharmaceutical Research Center, College of Pharmaceutical Sciences, Manipal- 576 104

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A simple, fast, precise and accurate reverse phase HPLC method was developed for the simultaneous estimation of paracetamol and rofecoxib in tablets. This method is based on using a Hypersil C_{18} column with a mobile phase of 20 mM phosphate buffer (pH 7.0±0.1) and acetonitrile in the ratio of 55:45 v/v. Valdecoxib was used as an internal standard. The retention times for paracetamol, rofecoxib and valdecoxib were 2.61, 10.09 and 12.31 min, respectively. The proposed method has also been validated. The method was found to be linear (r>0.999), precise (RSD: 0.82% for paracetamol, 0.42% for rofecoxib), accurate (mean percentage recovery yields: 99.3% for paracetamol and 98.4% for rofecoxib) and selective. Due to these attributes, the proposed method could be used for routine quality control analysis of these drugs in combined dosage forms.

Rofecoxib (ROF) is a new non-steroidal antiinflammatory drug. Chemically rofecoxib is known as 4-(4-methanesulphonylphenyl) 3-phenyl-5H-furan-2-one. Paracetamol (PAR), chemically 4-hydroxyacetanilide, is a centrally and peripherally acting non-opioid analgesic and antipyretic. A combination of these drugs, PAR (500 mg) and ROF (25 mg) is available commercially (Rofiz Plus) for the treatment of pain and spasm associated with musculoskeletal disorders. PAR is official in IP[1], BP[2] and USP[3], whereas ROF is not official in any Pharmacopoeias. Many HPLC methods have been described in the literature for the determination of PAR[4,5] and ROF[6-8] individually. However, there is no HPLC method reported for the simultaneous determination of these drugs either as active pharmaceutical ingredients or from combined dosage forms. The present work describes a simple, precise and accurate reverse phase HPLC method for simultaneous estimation of ROF and PAR in tablets.

PAR was obtained from Karnataka Antibiotics and Pharmaceuticals Limited, Bangalore. ROF and valdecoxib were obtained from Unichem laboratories, Mumbai as gift samples. Sodium dihydrogen orthophosphate, disodium hydrogen phosphate of AR grade and acetonitrile of HPLC grade were supplied by S. D. Fine Chemicals, Mumbai. Water HPLC grade was obtained from a Milli-Q RO water purification system. A gradient high-pressure liquid chromatograph (Shimadzu HPLC Class VP series) with two LC-10AT VP pumps, variable wavelength programmable UV/Vis detector SPD-10AVP, SCL-10AVP system controller (Shimadzu) and operating software Shimadzu Class VP version 6.12 SP2 data station was used for the analysis.

The method was carried out on a Hypersil C-18 (250 mmx4.6 mm, id- 5 µ) column as a stationary phase and acetonitrile:20 mM phosphate buffer (pH 7.0±0.1, adjusted with phosphoric acid) in the ratio of 45:55 v/v as the mobile phase at the flow rate of 1 ml/min. The mobile phase was filtered through a 0.45 µ membrane filter and degassed before analysis. A Rheodyne 7725 injector with a 20 µl loop was used for the injection of samples. Detection was done at 254 nm and separation was carried out at the room temperature of about 20°. Standard stock solution of ROF (100 µg/ml) was prepared in methanol. Stock solutions of PAR and valdecoxib were prepared in methanol and buffer (1:1 v/v). From the standard stock solutions, mixed standard solution was prepared containing 0.5 µg/ml of ROF, 10 µg/ml of PAR and 10 µg/ml of valdecoxib as internal standard was used as standard preparations.

Ten marketed tablets, each containing 25 mg of ROF

***For correspondence**
E-mail: n.udupa@cops.manipal.edu

and 500 mg of PAR (Rofiz plus, Wockhardt Limited, Mumbai) were weighed and finely powdered. A quantity of powder equivalent to 5 mg of ROF and 100 mg of PAR was weighed accurately and transferred to a 100 ml volumetric flask. Methanol was added, about 50 ml and sonicated it for 20 min and 10 ml of 1 mg/ml of VAL was added and the flask made up the volume up to 100 ml with methanol. Further dilutions of 10 times were made to get a concentration of 0.5 µg/ml of ROF, 10 µg/ml of PAR and 10 µg/ml valdecoxib (theoretical value). The content was vortexed and then filtered through 0.45 µ syringe filter. The resultant mixed standard solution was injected in triplicate into the liquid chromatograph. The ratio of peak area of drug to that of internal standard was calculated. The mixed standard solution was subjected to proposed HPLC method of analysis for finding out intra and interday variations. The validation of the proposed method was done as per the ICH guidelines[9]. The recovery studies were carried out by adding known amount of standard drug to the pre-analyzed samples and subjecting them to the proposed HPLC method of analysis. The precision of the method is studied by making six injections of standard solution.

The present study was carried out to develop a simple and rapid HPLC method for the simultaneous estimation of PAR and ROF using most widely used Hypersil C-18 column. The UV detection was carried out at 254 nm as ROF and PAR showed maximum absorbance around that wavelength. The UV absorption of ROF was more than PAR at this wavelength and because of which it was possible to have appreciable response of ROF (0.5 µg/ml) and also the detector response was not saturated for PAR (10 µg/ml). Thus it was possible to analyze both the drugs in single chromatogram. The typical chromatogram of PAR and ROF with the internal standard valdecoxib in the formulation is presented in fig. 1. The retention time of PAR, ROF and valdecoxib was found to be 2.51, 10.01 and 12.26 min, respectively. The assay concentration of 0.5 µg/ml ROF and 10 µg/ml PAR was selected according to the labeled claim. The peaks were well resolved and the capacity factor between PAR and ROF was found to be 10.88. The separation was interrupted by the presence of excipients peak which eluted at 7.4 min. Hence the peaks of PAR and ROF were well resolved with high capacity factor. The peak of internal standard was 12.31 min and it was well resolved from the other analytes. The asymmetry factor of all the peaks was lesser than 1.20 and it showed that all peaks are symmetrical in shape. The precision of the proposed method was lesser than 2% for all the three drugs and there was good

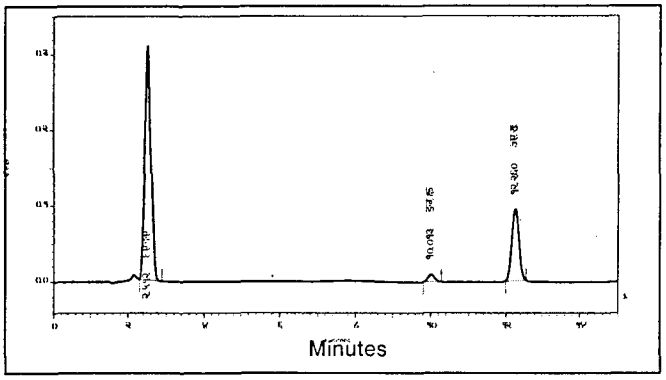

Fig. 1: Typical chromatogram of sample solution

Typical chromatogram of the sample solution containing paracetamol and rofecoxib at the retention time of 2.51 and 10.01 min. Valdecoxib (internal standard) was eluted at 12.26 min.

repeatability of the proposed method. The Co-efficient of variance was found to be 0.82% for PAR, 0.42% for ROF and 0.81% of valdecoxib, which was lower value when compared to acceptable co-efficient of variance, 2 % and it showed that the method was highly precise. The regression equation was found to be linear in the 70 to 130% of assay concentration. Accuracy of method was calculated by % mean recovery studies (n=3). Recovery studies were carried out by the addition of standard analyte to the analyzed sample. The concentration of standard spiked to the sample was 0.35-0.65 µg/ml of ROF and 7.0-13.0 µg/ml of PAR. The recovery studies are showed in the Table 1. The mean percentage recovery was found to be 98.4 for ROF and 99.3 for PAR. Assay of the combination in tablet dosage form were found to be 97.4% of ROF, 98.5% of PAR. The estimated amount was within the acceptable limits of the labeled claim of the formulation. The system suitability studies were carried out and the capacity factor of ROF and PAR was found to be 10.9 and 1.96, respectively. The tailing factor was 1.05 for ROF and 1.12 for PAR.

The proposed HPLC method was simple and precise because of the commonly used buffer, easier extraction procedures and short run time. High percentage of recovery shows that the method is free from the interferences of the excipients used in the formulations. The proposed method is highly accurate which showed good recovery of the drug samples and there was no interference from the excipients at the retention time of all three drugs, which showed that it was specific and the analysis was less time consuming. The proposed method can be used in routine quality control of

TABLE 1: RECOVERY EXPERIMENTS

Drug	Amount added (μg) n=3	Amount recovered (μg) n=3	Percent recovery	Average percent recovery
	0.3591	0.3538	98.6	
Rofecoxib	0.5052	0.4959	98.2	98.4
	0.6580	0.6468	98.3	
	7.0801	7.0701	99.9	
Paracetamol	10.1202	9.9961	98.8	99.3
	13.1312	13.0223	99.2	

Recovery experiment data for ROF and PAR showing the amounts of drug added and recovered from sample solution at each level (n=3), percentage recovery and the average percentage recovery. ROF stands for Rofecoxib and PAR stands for Paracetamol

combined dosage form containing rofecoxib and paracetamol in tablets.

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Spectrophotometric Determination of Repaglinide in Tablet Dosage Forms

S. K. JAIN, G. P. AGRAWAL AND N. K. JAIN*

Pharmaceutics Research Laboratory, Department of Pharmaceutical Sciences,
Dr. Hari Singh Gour University, Sagar-470 003

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A simple visible spectrophotometric method has been developed for the estimation of repaglinide in tablet formulations. Beer's law is obeyed in the concentration range of 5-50 μg/ml of repaglinide. The method is simple, precise and accurate for pure analyte with recovery of 99.5-99.9%. It does not require any separation of soluble excipients present in tablets, as they do not interfere in the estimation.

***For correspondence**
E-mail: jnarendr@yahoo.co.in